REMARKS

A. Claim Amendments

For clarity, Claim 2 has been amended to specify that the proteins whose cleavage into soluble form is referenced (TNFα and TNFα lacking the TACE metalloproteinase recognition site spanning the valine 77 to the proline 88 of TNFα) are human TNFα, as described in, for example, paragraphs 0008 and 0082, and referenced in the experiments described at paragraphs 0111 to 0113, and Figures 3 through 5. Extraneous hyphens in the cell line names recited in Claim 2 have also been deleted.

No new matter has been added to the application by the amendment and newly added claims, entry of which is therefore respectfully requested.

B. Response to Rejection of Claims 2-4, 8, 11-12, 27-29, 32-41, 68 and 76-79 under 35 USC 112, First Paragraph (written description).

The listed claims are rejected on the basis that the Specification lacks essential matter in not reciting the entire amino acid sequence of the deleted mmp recognition site and/or the amino acid sequence of TNFα. To the extent that the rejection requires more clarity about the identity of the particular TNFα containing the recognition sequence recited as a reference in Claim 2, the present amendment to the claim provides it. In particular, it is now specified that the reference is to *human* TNFα molecules and that the putatively deleted sequence is the entire "TACE mmp recognition sequence" spanning from Val77 to Pro88 in human TNFα.

The amino acid sequences for human TNFα and the Val77 to Pro88 span were well known prior to the filing of the patent application. The molecules are clearly described in the Specification to an extent allowing one of ordinary skill in the art to readily and unambiguously identify them. For example, the reference molecules which produce more soluble TNFα than the chimeric molecules of the invention are described in the Specification as human pro-TNFα lacking a

specified mmp recognition sequence (for the TACE mmp) and human TNFα at, for example, paragraphs 0007 and 0008, which read:

> "There are two bioactive forms of TNF.alpha.. One form is membrane-integrated (mTNF.alpha.), also referred to as pro-TNF.alpha... [*paragraph 0007*].
>
> A matrix metalloproteinase (mmp) called TACE (for TNF-alpha converting enzyme) has been shown to release the soluble form of TNF.alpha. (Black et al, Nature, 385:729-733, 1997 and Moss et al, Nature, 385:733-736, 1997). TACE has been found to release sTNF.alpha. by cleaving pro-TNF.alpha. between amino acid residues alanine76 and valine77. Moreover, this cleavage is dependent on an approximately **12 amino acid mmp recognition sequence spanning valine77 to proline88** (Decoster et al, J Biol Chem, 270:18473-18478, 1995 and Tang et al, Biochemistry, 35:8226-8233, 1996) since deletion of 9 to 12 amino acids of this mmp recognition site inhibited the cleavage of the parent TNF.alpha. molecule (Decoster et al, J Biol Chem, 270:18473-18478, 1995 and Perez et al, Cell, 63:251-258, 1990). However, deletion of this cleavage site does not necessarily completely abrogate sTNF.alpha. generation due to the existence of multiple cleavage sites in TNF.alpha. (Mueller et al, J Biol Chem, 274:38112-38118, 1999)." [*paragraph 0008, emphasis added*].

The amino acid sequence of TNFα has been known to the art since the early 1990s, including the "12 amino acid mmp recognition sequence spanning valine77 to proline88" as taught above and now recited in Claim 2. There is no question that one of ordinary skill in the art would know, or could readily obtain, the structure of the recited polypeptides with very little effort—consulting the published art referenced in the patent application would do it, as would a quick search of any protein database, such as the protein bank on the National Institutes of Health's PubMed, which includes the amino acid sequence for human TNFα as NP_000585.2 (see, enclosed Attachment A).

The complete sequence of TNFα and the TACE recognition site are therefore not essential matter which must be disclosed in the Specification to fully describe the invention and enable the art to practice it. To the contrary, it is axiomatic that one need not disclose details of structures which are well-known in the art to meet the requirements of Section 112, first paragraph (see, e.g., *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed.Cir.1986) ("a patent need not teach, and preferably omits, that which is known in the art)).

Reconsideration and withdrawal of the Section 112, first paragraph (written description) rejection of the claims is therefore respectfully requested.

C. Response to Rejection of Claims 2-4, 8, 11-12, 27-29, 32-41, 68 and 76-79 under 35 USC 112, First Paragraph (written description).

The listed claims are rejected on the basis that the HeLa, 293, A549, COLO205, HCT-15, BT-20 and HT1080 cells/cell lines must be known to and readily available to the public or deposited.

The cells recited are extremely common tools used in cancer research, with many of them being publicly available since the 1950s, and all being commercially available since well prior to the filing date of the instant application (see, for example, Attachment B, pages from the American Type Culture Collection's cell line catalog listing for sale: HeLa cells as ATCC CCL-2, 293 cells as CCL-1573, HT 1080 cells as CCL-121, A549 cells as CCL-185, COLO 205 cells as CCL-222, HCT 15 cells as CCL-225, and BT 20 cells as HTB-19). One of ordinary skill in the art would immediately recognize the references in the claims to these cell lines and would either be likely to already have them on hand, or be able to readily obtain them.

Reconsideration and withdrawal of the Section 112, first paragraph (written description) rejection of the claims is therefore respectfully requested.

D. Supplemental Response to the Double-Patenting Rejections

Claims 2-4, 8, 11-12, 27-29, 32-41, 68, and 76-79 are objected to on the basis that "Applicant's amendment, filed 7/31/2009, does not address the issue of commonly owned at the time the invention was made." In response, it is confirmed as follows:

As to commonly assigned USSN 11/015,117 (now US Pat. No. 7524944), the invention claimed therein was made after the invention presently claimed. At that time, and at all times since, the invention of the '944 Patent and the presently claimed invention have been assigned to and/or subject to an obligation on the part of all inventors to assign to the present Assignee, the Regents of the University of California (see, confirmatory assignments recorded at reel/ frame 021549/0140 ['944 Patent] and reel/frame 012962/0392 [present application]).

As to commonly assigned US Pat. No. 7070771, the invention claimed therein was made prior to the invention presently claimed. At that time, and at all times since, the invention of the '771 Patent and the presently claimed invention have been assigned to and/or subject to an obligation on the part of all inventors to assign to the present Assignee, the Regents of the University of California (see, confirmatory assignments recorded at reel/ frame 0158921 ['771 Patent] and reel/frame 012962/0392 [present application]).

Statements under 37 CFR Section 3.73(b) confirming the above chains of title are already of record in the previously submitted and recorded terminal disclaimers viz the '944 and '771 Patents.

Reconsideration of the common ownership objection is therefore respectfully requested.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

No fee is deemed necessary in connection with the filing of this paper. However, the Commissioner is hereby authorized to charge any other fees that may be due in connection with the filing of this paper, or credit any overpayment to Deposit Account No. 07-1896.

Respectfully submitted,

Date: March 1, 2010

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Registration No. 34,842
Telephone: (858) 677-1423
Facsimile: (858) 677-1465

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4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer Number 28213

Attachments:
Attachment A (Pages from the National Institutes of Health's PubMed protein bank)
Attachment B (Pages from the American Type Culture Collection's cell line catalog)

Attachment A

NCBI Reference Sequence: NP_000585.2

tumor necrosis factor alpha [Homo sapiens]

Comment Features Sequence

```
LOCUS       NP_000585               233 aa            linear   PRI 07
-FEB-2010
DEFINITION  tumor necrosis factor alpha [Homo sapiens].
ACCESSION   NP_000585
VERSION     NP_000585.2  GI:25952111
DBSOURCE    REFSEQ: accession NM_000594.2
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates;
Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 233)
  AUTHORS   Skibola,C.F., Bracci,P.M., Nieters,A., Brooks-Wilson,A.,
de
            Sanjose,S., Hughes,A.M., Cerhan,J.R., Skibola,D.R.,
Purdue,M.,
            Kane,E., Lan,Q., Foretova,L., Schenk,M., Spinelli,J.J.,
            Slager,S.L., De Roos,A.J., Smith,M.T., Roman,E., Cozen,W.,
            Boffetta,P., Kricker,A., Zheng,T., Lightfoot,T., Cocco,P.,
            Benavente,Y., Zhang,Y., Hartge,P., Linet,M.S., Becker,N.,
            Brennan,P., Zhang,L., Armstrong,B., Smith,A., Shiao,R.,
Novak,A.J.,
            Maynadie,M., Chanock,S.J., Staines,A., Holford,T.R.,
Holly,E.A.,
            Rothman,N. and Wang,S.S.
  TITLE     Tumor necrosis factor (TNF) and lymphotoxin-alpha (LTA)
            polymorphisms and risk of non-Hodgkin lymphoma in the
InterLymph
            Consortium
  JOURNAL   Am. J. Epidemiol. 171 (3), 267-276 (2010)
   PUBMED   20047977
  REMARK    GeneRIF: Meta-analysis of gene-disease association. (HuGE
            Navigator)
REFERENCE   2  (residues 1 to 233)
  AUTHORS   La Manna,G., Cappuccilli,M.L., Cianciolo,G., Conte,D.,
Comai,G.,
            Carretta,E., Scolari,M.P. and Stefoni,S.
  TITLE     Cardiovascular Disease in Kidney Transplant Recipients:
The
            Prognostic Value of Inflammatory Cytokine Genotypes
```

JOURNAL Transplantation (2010) In press
PUBMED 20061995
REMARK GeneRIF: Observational study of gene-disease association.
(HuGE Navigator)
Publication Status: Available-Online prior to print
REFERENCE 3 (residues 1 to 233)
AUTHORS Welsby,I.J., Podgoreanu,M.V., Phillips-Bute,B., Morris,R., Mathew,J.P., Smith,P.K., Newman,M.F., Schwinn,D.A. and Stafford-Smith,M.
CONSRTM Perioperative Genetics and Safety Outcomes Study (PEGASUS) Investigative Team
TITLE Association of the 98T ELAM-1 Polymorphism with Increased Bleeding After Cardiac Surgery
JOURNAL J. Cardiothorac. Vasc. Anesth. (2010) In press
PUBMED 20056442
REMARK GeneRIF: Observational study of gene-disease association.
(HuGE Navigator)
Publication Status: Available-Online prior to print
REFERENCE 4 (residues 1 to 233)
AUTHORS Ghosh,J., Joshi,G., Pradhan,S. and Mittal,B.
TITLE Investigation of TNFA 308G > A and TNFB 252G > A polymorphisms in genetic susceptibility to migraine
JOURNAL J. Neurol. (2009) In press
PUBMED 20035431
REMARK GeneRIF: Observational study of gene-disease association.
(HuGE Navigator)
Publication Status: Available-Online prior to print
REFERENCE 5 (residues 1 to 233)
AUTHORS Menegatti,E., Davit,A., Francica,S., Berardi,D., Rossi,D., Baldovino,S., Tovo,P.A., Sena,L.M. and Roccatello,D.
TITLE Genetic factors associated with rheumatoid arthritis and systemic vasculitis: Evaluation of a panel of polymorphisms
JOURNAL Dis. Markers 27 (5), 217-223 (2009)
PUBMED 20037209
REMARK GeneRIF: Observational study of gene-disease association.
(HuGE Navigator)
REFERENCE 6 (sites)
AUTHORS Mohan,M.J., Seaton,T., Mitchell,J., Howe,A., Blackburn,K., Burkhart,W., Moyer,M., Patel,I., Waitt,G.M., Becherer,J.D., Moss,M.L. and Milla,M.E.
TITLE The tumor necrosis factor-alpha converting enzyme (TACE): a unique metalloproteinase with highly defined substrate selectivity
JOURNAL Biochemistry 41 (30), 9462-9469 (2002)
PUBMED 12135369
REMARK Erratum:[Biochemistry. 2003 Sep 23;42(37):11092]
REFERENCE 7 (sites)
AUTHORS English,W.R., Puente,X.S., Freije,J.M., Knauper,V., Amour,A., Merryweather,A., Lopez-Otin,C. and Murphy,G.
TITLE Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis

See reference m
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factor-alpha convertase activity but does not activate pro -MMP2
JOURNAL J. Biol. Chem. 275 (19), 14046-14055 (2000)
PUBMED 10799478
REFERENCE 8 (sites)
AUTHORS Roghani,M., Becherer,J.D., Moss,M.L., Atherton,R.E., Erdjument-Bromage,H., Arribas,J., Blackburn,R.K., Weskamp,G., Tempst,P. and Blobel,C.P.
TITLE Metalloprotease-disintegrin MDC9: intracellular maturation and catalytic activity
JOURNAL J. Biol. Chem. 274 (6), 3531-3540 (1999)
PUBMED 9920899
REFERENCE 9 (sites)
AUTHORS Pocsik,E., Duda,E. and Wallach,D.
TITLE Phosphorylation of the 26 kDa TNF precursor in monocytic cells and in transfected HeLa cells
JOURNAL J. Inflamm. 45 (3), 152-160 (1995)
PUBMED 8597870
REFERENCE 10 (residues 1 to 233)
AUTHORS Buonaguro,L., Barillari,G., Chang,H.K., Bohan,C.A., Kao,V., Morgan,R., Gallo,R.C. and Ensoli,B.
TITLE Effects of the human immunodeficiency virus type 1 Tat protein on the expression of inflammatory cytokines
JOURNAL J. Virol. 66 (12), 7159-7167 (1992)
PUBMED 1279199
REFERENCE 11 (residues 1 to 233)
AUTHORS Zhang,X.M., Weber,I. and Chen,M.J.
TITLE Site-directed mutational analysis of human tumor necrosis factor-alpha receptor binding site and structure-functional relationship
JOURNAL J. Biol. Chem. 267 (33), 24069-24075 (1992)
PUBMED 1331108
REFERENCE 12 (residues 1 to 233)
AUTHORS Stevenson,F.T., Bursten,S.L., Locksley,R.M. and Lovett,D.H.
TITLE Myristyl acylation of the tumor necrosis factor alpha precursor on specific lysine residues
JOURNAL J. Exp. Med. 176 (4), 1053-1062 (1992)
PUBMED 1402651
REFERENCE 13 (sites)
AUTHORS Stevenson,F.T., Bursten,S.L., Locksley,R.M. and Lovett,D.H.
TITLE Myristyl acylation of the tumor necrosis factor alpha precursor on specific lysine residues
JOURNAL J. Exp. Med. 176 (4), 1053-1062 (1992)
PUBMED 1402651
REFERENCE 14 (residues 1 to 233)
AUTHORS Spriggs,D.R., Deutsch,S. and Kufe,D.W.
TITLE Genomic structure, induction, and production of TNF-alpha
JOURNAL Immunol. Ser. 56, 3-34 (1992)
PUBMED 1550865
REMARK Review article
REFERENCE 15 (residues 1 to 233)
AUTHORS Pryke,A.M., Duggan,C., White,C.P., Posen,S. and Mason,R.S.
TITLE Tumor necrosis factor-alpha induces vitamin D-1-hydroxylase activity in normal human alveolar macrophages
JOURNAL J. Cell. Physiol. 142 (3), 652-656 (1990)
PUBMED 1690216
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The

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Nucleotide
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PubMed
PubMed (Rel
PubMed (wei
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Taxonomy
UniGene
LinkOut

reference sequence was derived from M10988.1 and BI908079.1.
On Nov 29, 2002 this sequence version replaced gi:10835155.

Summary: This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine. [provided by RefSeq].

Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Entrez Gene record to access additional publications.

FEATURES Location/Qualifiers

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Attachment B

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Organism:	*Homo sapiens* (human)		Technical Si
Morphology:	epithelial		Related Cell
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Isolation:	**Isolation date:** July, 1972		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Virus Susceptibility:	Human poliovirus 1 RD-114 Feline Feline leukemia virus Vesicular stomatitis virus		
Tumorigenic:	Yes		
Reverse Transcript:	negative		
Oncogene:	ras +		

DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 12,14 D16S539: 9,12 D5S818: 11,13 D7S820: 9,10 THO1: 6 TPOX: 8 vWA: 14,19
Cytogenetic Analysis:	modal number = 46; range = 44 to 48. Pseudodiploidy was frequently noted. About 40% of the cells had rearranged karyotypes with an extra E-group chromosome and a group C chromosome, probably chromosome 11, was missing.
Isoenzymes:	G6PD, B
Age:	35 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells contain an activated N-ras oncogene.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:8 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020

References:

22147: Chen TR, et al. Intercellular karyotypic similarity in near-diploid cell lines of human tumor origins. Cancer Genet. Cytogenet. 10: 351-362, 1983. PubMed: 6652615
23071: Geiser AG, et al. Suppression of tumorigenicity in human cell hybrids derived from cell lines expressing different activated ras oncogenes. Cancer Res. 49: 1572-1577, 1989. PubMed: 2647289
23393: Rasheed S, et al. Characterization of a newly derived human sarcoma cell line (HT-1080). Cancer 33: 1027-1033, 1974. PubMed: 4132053
25969: Adams RA, et al. Direct implantation and serial transplantation of human acute lymphoblastic leukemia in hamsters, SB-2. Cancer Res. 28: 1121-1125, 1968. PubMed: 4872716
26035: . . Proc. Am. Assoc. Cancer Res. 8: 1, 1967.
32289: Hu M, et al. Purification and characterization of human lung fibroblast motility-stimulating factor for human soft tissue sarcoma cells: identification as an NH2-terminal fragment of human fibronectin. Cancer Res. 57: 3577-3584, 1997. PubMed: 9270031
32370: Ilda A, et al. Inducible gene expression by retrovirus-mediated transfer of a modified tetracycline-regulated system. J. Virol. 70: 6054-6059, 1996. PubMed: 8709228
32531: Brenneman M, et al. Stimulation of intrachromosomal homologous recombination in human cells by electroporation with site-specific endonucleases. Proc. Natl. Acad. Sci. USA 93: 3608-3612, 1996. PubMed: 8622983
33061: Seiffert D. Hydrolysis of platelet vitronectin by calpain. J. Biol. Chem. 271: 11170-11176, 1996. PubMed: 8626663
33152: Hocking AM, et al. Eukaryotic expression of recombinant biglycan. J. Biol. Chem. 271: 19571-19577, 1996. PubMed: 8702651

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Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA		
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Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]		
Receptors:	vitronectin, expressed		
Tumorigenic:	Yes		

DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t (8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der (1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Age:	fetus
Comments:	Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768] The line is excellent for titrating human adenoviruses. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406] The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended. 6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.
	Subcultivation Ratio: 1:10 to 1:20 weekly. **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006
derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D

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Isolation:	**Isolation date:** 1972		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Reverse Transcript:	negative		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 11 D16S539: 11,12 D5S818: 11 D7S820: 8,11 THO1: 8,9.3 TPOX: 8,11 vWA: 14		

Cytogenetic Analysis: This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6) (q11;q27); ?del(6) (p23); del(11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.

Isoenzymes: G6PD, B

Age: 58 years

Gender: male

Ethnicity: Caucasian

Comments: This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. [23218] Further studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway. [58030]
The cells are positive for keratin by immunoperoxidase staining.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
   Cultures can be established between 2 X 10(3) and 1 X 10(4) viable cells/cm2. Do not exceed 7 X 10(4) cels/cm2.
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 6 X 10(3) and 6 X 10(4) cell/cm2.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: about 22 hours

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

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Applications:	transfection host (Roche FuGENE® Transfection Reagents)		
Tumorigenic:	Yes		
Reverse Transcript:	negative		

DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 8,11 D16S539: 12,13 D5S818: 13 D7S820: 10,12 THO1: 7,9.3 TPOX: 8,11 vWA: 18,19
Cytogenetic Analysis:	This is a quasidiploid human cell line with the modal number 46 occurring in 76% of cells (range = 41 to 47 for 50 metaphases).The rate of polyploidy was 5.1%. The karyotype of the line 46, XY, -8,-11, -17, t (8:17)(p23:q21), inv(11)(p15.3q13.1). The Y chromosome was slightly longer than N22, and had a large segment of heterochromatic, fluorescent distal q arms.
Isoenzymes:	ES-D, 2 G6PD, B PEP-D, 1 PGD, A PGM1, 1-2 PGM3, 1
Gender:	male
Comments:	Evidence from DNA fingerprinting indicates that this line and DLD-1 (ATCC CCL-221) are derived from the same individual; however, isoenzymology and cytogenetic data leave some doubt. HCT-15 cells are CSAp negative (CSAp-). The cells are positive for keratin by immunoperoxidase staining.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:10 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium, 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020

References:

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Isolation:	**Isolation date:** 1958		
Tumorigenic:	Yes		
Reverse Transcript:	negative		
Antigen Expression:	HLA A1, Bw16 (+/-)		
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 11 D16S539: 11,14 D5S818: 12 D7S820: 10 THO1: 7,9.3 TPOX: 11 vWA: 16,17		

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Cytogenetic Analysis:	Normal chromosomes N3, N4, N9, N13, N14, and X may be absent. The markers der(11)t(11;?)(q25;?) (M1); der(1)t(1;3)(p22?;p13?) (M2); and der(2)t(2;?) (q37;?) (M5) were detected by W.A. Nelson-Rees, et al., Int. J. Cancer 16: 74-85, 1975.
Isoenzymes:	AK-1, 1-2 ES-D, 1 G6PD, B GLO-I, 1-2 PGM1, 1 PGM3, 1
Age:	74 years
Gender:	female
Ethnicity:	Caucasian
Comments:	The cells express the WNT3 and the WNT7B oncogenes [PubMed: 8168088]. This breast tumor line was established by E.Y. Lasfargues and L. Ozzello in 1958 by isolation and cultivation of cells spilling out of the tumor when it was cut in thin slices. A mycoplasma contaminant was discovered and eliminated early in 1972. Growth of BT-20 cells is inhibited by tumor necrosis factor alpha (TNF alpha). BT-20 cells are negative for estrogen receptor, but do express an estrogen receptor mRNA that has deletion of exon 5.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gentle pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020

References:

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